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### Source of Boron in Curcuma for Burn Symptoms at Leaf Margins

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## Source of Boron in *Curcuma* for Burn Symptoms at Leaf Margins

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### ABSTRACT

This study was initiated to investigate the source of boron (B) accumulated in margins of old leaves of *Curcuma* 'Chiangmai University Pride' ('CMU Pride'). Nutrient elements were analyzed from different parts of the rhizome before potting and from whole rhizomes at harvest, and different leaf locations of young and old leaves during forcing in coarse sand medium. Plants were fertilized with solutions lacking B during the forcing period. Leaf-margin burn symptoms in old leaves of 'CMU Pride' could result from the combination of high levels of B and low total N levels. High levels of manganese (Mn) may not be involved in leaf margin-burn symptoms. Boron accumulated in the outer region of the old leaves could result from following evaporation of guttated water droplets containing dissolved B. High levels of B in the edge of the old leaves may not be transported from the rhizomes.

**Keywords:** boron toxicity, foliar analysis, rhizome position, physiological disorder

### INTRODUCTION

Genotypes of *Curcuma* spp. (*Zingiberaceae*), native to tropical Asia, have been cultivated as ornamentals because they have large, showy, concave, or hooded bracts (Bailey, 1925). *Curcuma* species or cultivars that have showy bracts and a long post-harvest vase-life are produced as commercial cut flowers, while some dwarf forms are used as potted plants (Lawson and Roh, 1992; Roh et al., 2006).

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General cultural information is available for *Curcuma alismatifolia* Gagnep. and *C. parviflora* Wall to produce cut flowers or potted plants (Kuehny et al., 2002; Roh and Lawson, 1993; Roh et al., 2006).

Older leaves often exhibit leaf-margin burn symptoms and this was correlated to high concentrations of boron (B), iron (Fe), and manganese (Mn) (Roh et al., 2006). These symptoms were observed in older leaves of 'CMU Pride' with broad leaves (Roh et al., 2006) but the leaf-margin burn symptoms were not observed in young leaves of 'CMU Pride', or *C. alismatifolia* with narrow leaves. Leaves with a high Mn and Fe content had severe leaf-margin burn symptoms. High levels of Mn may not be responsible for leaf-margin burn symptoms because interveinal chlorosis symptoms, typical of Mn toxicity (Joiner et al., 1981; Mastalerz, 1977) were not present.

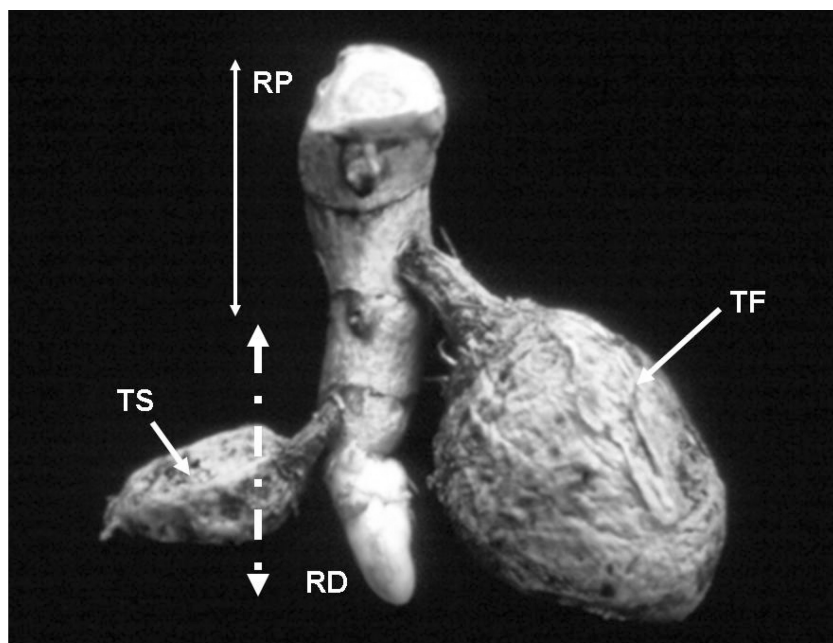
It has not been investigated whether the source of B responsible for leaf-margin burn symptoms observed in old leaves of *Curcuma* species with broad leaves is supplied from the growing medium, rhizomes, or leaf guttation. The active or passive uptake of B is not well understood (Tanaka, 1967). Boron is relatively immobile and upper plant parts have a high content, which could be affected by the rate of transpiration (Michael et al., 1969). Boron accumulates in the tip or margin of the leaves (Jones, 1970) and may cause toxic effects (Oertli, 1962). Boron uptake occurs via passive diffusion in *Arabidopsis thaliana* mutant and substantial retention of B was observed at xylem loading at high B levels (Dannel et al., 2002; Takano et al., 2002). The objectives of this study were (a) to investigate the source of accumulated B in the affected part of the leaves and (b) determine the relationship between leaf margin-burn symptoms and nutrient element content of leaf tissue.

## MATERIALS AND METHODS

### Plant Material and Cultural Practices

Rhizomes of *C. sp.* 'CMU Pride', *C. alismatifolia* 'Pink', and *C. 'Waxy Candle'* were stored at 20°C until potting on 11 May. Before potting, clumps of rhizome were separated and uniform rhizome pieces were collected (Figure 1). Tissue samples were obtained from the proximal end and distal end of rhizome and also from turgid and semi-shriveled tuberous root. Dried and shriveled tuberous roots (not shown in Figure 1) and fibrous roots formed from the rhizomes were also collected for tissue analysis as described (Roh et al., 2006). After forcing, rhizomes with tuberous roots that received nutrient solutions were analyzed.

One rhizome with three rhizomes tips with a minimum of four tuberous roots attached to each rhizome tip was planted per 15-cm pot filled with coarse sand. Sand was washed with tap water to remove soil and organic matter before filling the pots. The sand had a media analysis of pH 6.9, 0.17 mmhos/cm soluble salts, 0.0% nitrate nitrogen, 0.8 ppm ammonium nitrogen,



**Figure 1.** Sampling of rhizome piece of *C. 'CMU Pride'*. Tissue samples were obtained from the proximal end (RP) and distal end (RD) of the rhizome and also from turgid (TF) and semi-shriveled (TS) tuberous roots. Severely shriveled and dried tuberous root and fibrous roots formed from the rhizomes are not shown.

1.65 ppm phosphorus (P), 4.41 ppm potassium (K), 17.2 ppm calcium (Ca), 3.3 ppm magnesium (Mg), 0.076 ppm boron (B), 0.21 ppm iron (Fe), 0.0005 ppm manganese (Mn), 0.026 ppm copper (Cu), 0.003 ppm zinc (Zn), 0.004 ppm molybdenum (Mo). The level of ammonium nitrogen, boron, and copper was close to the low side of the normal range, which is 0–20 ppm, 0.05 ppm, and 0.0001–0.5 ppm, respectively. The level of B, Fe, and Mn in tap irrigation water was 0.000, 0.009, and 0.005 ppm, respectively, and in distilled water were 0.000, 0.000, and 0.002 ppm, respectively.

Plants were grown in a greenhouse with minimum air temperatures of 25°C/21°C (day/night), although temperatures varied by +5°C during summer. There were ten rhizomes per species or cultivar. During forcing, stock solutions of major nutrients and of micro nutrients were diluted and mixed, and then plants were fertilized every Monday with a nutrient solution containing 4 g L<sup>-1</sup> of calcium nitrate [Ca (NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O], 2 g L<sup>-1</sup> of potassium nitrate (KNO<sub>3</sub>), 4 g L<sup>-1</sup> magnesium sulfate (MgSO<sub>4</sub> 7H<sub>2</sub>O), 0.02 g L<sup>-1</sup> iron (Fe)-ethylenediaminetetraacetic acid (EDTA), and 2 g L<sup>-1</sup> potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>); micronutrients lacking boron [0.5 g L<sup>-1</sup> manganese

chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ),  $0.05 \text{ g L}^{-1}$  zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ),  $0.02 \text{ g L}^{-1}$  copper sulfate ( $\text{CuSO}_4$ ),  $0.01 \text{ g L}^{-1}$  sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ )]. Greenhouse light irradiance was maintained at  $24\text{--}28 \mu\text{moles m}^{-2} \text{ s}^{-1}$  between 0700–1700 h by shade cloth, or by supplementary high-intensity discharge lighting from high-pressure sodium lamps.

### Sampling for Tissue Analysis and Data Analysis

The first inflorescence reached anthesis 5 September. On 8 October, leaf samples were collected from *C. 'CMU Pride'*, *C. alismatifolia*, and *C. 'Waxy Candle'* for tissue analysis. Samples were collected from the seventh young leaf counted from the bottom which is close to the inflorescence and did not show any visual leaf-margin burn symptoms, from the second oldest leaf which is distant from the first inflorescence and showed severe leaf-margin burn symptoms, and a young expanding leaf from a newly developing shoot without visible inflorescence with no symptoms. Leaves were divided into distal end and proximal end, and then further divided into an outer and inner part, resulting in four samples per leaf (refer to Figure 1 of Roh et al., 2006). Two replicates of leaf tissues, growing medium, and irrigation water were analyzed at J. R. Peters Laboratory (then Grace-Sierra Testing Laboratory, Allentown, PA). Data were subjected to the analysis of variance (ANOVA), and mean comparisons were done by Tukey's  $\omega$ -procedures with the honestly significant difference (hsd) value at 1% (Michigan State University, 1989).

## RESULTS

### Analysis of Nutrients in Growing Medium and Plant Tissues at Potting

At potting of 'CMU Pride', the level of N in proximal end and distal end of the rhizome, turgid tuberous roots and semi-shriveled tuberous roots (Figure 1) and also fibrous roots and severely shriveled tuberous roots were lower than the normal range (2.00–5.50%) (Table 1). Considering that the normal range of the level of B, Fe, and Mn in most floral crops is 30–100 ppm, 50–300 ppm, and 50–500 ppm, respectively, the level in all parts of rhizomes were lower than normal. The level of these elements in the entire rhizome pieces analyzed after forcing was also low. The level of B did not differ among parts of the rhizome before potting or for combined rhizomes after forcing. The level of other minor elements such as Cu, Zn, and Mo was at the lower end of the normal range or low in all parts of the rhizomes at potting and after harvest. The level of P, K, Ca, and Mo was in the normal range, but Cu and Zn were low. In leaves

Table 1  
Nutrient analysis in different parts of 'CMU Pride' rhizomes at potting and after forcing

	At potting					After forcing	hsd <sup>3</sup>
	Nutrient fibrous element roots	Severely shriveled tuberous roots <sup>1</sup>	Slightly shriveled tuberous roots (TS)	Turgid tuberous roots (TF)	Distal end of rhizomes (RD)	Proximal end of rhizomes (RP)	
Nitrogen (%)	1.01	1.63	1.51	1.61	2.47	2.30	0.48
Boron (ppm)	9.00	4.76	4.60	3.68	4.22	3.37	2.35
Iron (ppm)	55.6	23.7	29.5	36.3	40.6	36.6	4.86
Manganese (ppm)	38.2	8.98	6.93	7.66	38.5	31.4	3.91

<sup>1</sup>Refer to Figure 1.

<sup>2</sup>Combined samples of TS, TF, RD, and RP.

<sup>3</sup>Honestly significant difference procedure.

of 'Waxy Candle', Cu, Zn, and Mo levels were low (data not presented). The level of other elements was similar to 'CMU Pride'.

### Symptom Expression and Tissue Analysis

'CMU Pride' and 'Waxy Candle' showed leaf-margin burn symptoms while *C. alismatifolia* did not. *Curcuma alismatifolia* did not even show leaf-margin burn symptoms two months after flowering (August 15). The levels of B, Fe, and Mn were 10.8 ppm, 40 ppm, and 61 ppm, respectively, in *C. alismatifolia*. Potassium (K) was in the normal range, 2.5–3.7%. The levels of Cu and Zn in *C. alismatifolia* were generally low and all other microelements fell into the normal range regardless of the position of leaves on the inflorescence and various regions in the leaf (data not presented).

The level of nitrogen (N) in old leaves formed in the first inflorescence was 1.2% to 1.7%. This was below the normal range and lower than N levels in young leaves formed in the first inflorescence or in the second inflorescence that fell into the normal range for N (2.0–3.0%). The level of B in the edge of the distal part of old leaves with severe leaf-burn symptoms in both 'CMU Pride' and 'Waxy Candle' was higher than the normal range, reaching to 112 ppm and 113 ppm, respectively (Table 2). The level of Fe and Mn was in the normal range, regardless of leaf age or region in a given leaf. The level of B and Mn in 'CMU Pride' was significantly influenced by leaf ages (young and old leaves formed in the old inflorescence and young leaves in the developing shoots) and the leaf all regions of the leaves. In 'Waxy Candle', only B level (113 ppm) in the outer part of the distal end of old leaves was above the normal range. However, N, Mn, and Fe levels were in the normal range (Table 2).

## DISCUSSION

### Symptom Expression and Tissue Analysis

Leaf-margin burn symptoms were observed only in *Curcuma* with broad leaves, as reported previously, not in *C. alismatifolia* with narrow leaves (Roh et al., 2006). The causes of leaf-margin burn symptoms have been considered to be the high B level, interactions between Mn, or Fe with B, high Mn level, or the ratio of Mn: Fe as discussed by Bachman and Halbrooks (1993). The low level of N in senescing old leaves from the first inflorescence, as evidenced by the low level of N in all regions of the leaf, suggested that leaf-margin burn symptoms could result from the combination of a low total N level and a high level of B. Manganese (Mn) may not be involved in leaf-margin burn symptoms because the Mn level was low in 'Waxy Candle' leaves at the outer part of the distal end of old leaves. The typical interveinal chlorosis in leaves with leaf-margin burn

Table 2  
Foliar analysis of 'CMU Pride' (CMU) and 'Waxy Candle' (WC)

Tissue sample <sup>1</sup>				N (%)		B (ppm)		Fe (ppm)		Mn (ppm)	
Inflorescence/leaf age (Ag-I/L)	Leaf end (Lend)	Leaf part (Lpart)		CMU	WC	CMU	WC	CMU	WC	CMU	WC
First I/Young L	Distal	Outer		2.9	— <sup>2</sup>	19 <sup>lo3</sup>	—	75	—	169	—
First I/Young L	Distal	Inner		2.8	—	22 <sup>lo</sup>	—	60	—	93	—
First I/Young L	Proximal	Outer		2.5	—	13 <sup>lo</sup>	—	60	—	57	—
First I/Young L	Proximal	Inner		2.3	—	12 <sup>lo</sup>	—	50	—	44	—
First I/Old L	Distal	Outer		1.7 <sup>lo</sup>	2.2	122 <sup>hi</sup>	113 <sup>hi</sup>	184	69	263	32
First I/Old L	Distal	Inner		1.7 <sup>lo</sup>	2.6	32	30	101	62	176	32
First I/Old L	Proximal	Outer		1.2 <sup>lo</sup>	2.5	9 <sup>lo</sup>	14 <sup>lo</sup>	34	55	134	42
First I/Old L	Proximal	Inner		1.2 <sup>lo</sup>	2.2	15 <sup>lo</sup>	13 <sup>lo</sup>	55	45	101	35
Second I/Young L	Distal	Outer		3.0	—	18 <sup>lo</sup>	—	69	—	81	—
Second I/Young L	Distal	Inner		2.9	—	16 <sup>lo</sup>	—	65	—	74	—
Second I/Young L	Proximal	Outer		2.7	—	12 <sup>lo</sup>	—	54	—	32 <sup>1</sup>	—
Second I/Young L	Proximal	Inner		2.0	—	11 <sup>lo</sup>	—	39	—	22 <sup>1</sup>	—

Level of significance									
Inflorescence /leaf age (Ag-I/L)	*** 4	—	***	—	***	***	—	***	—
Leaf end (Lend)	***	**	***	***	***	***	***	***	ns
Leaf part (Lpart)	***	ns	***	**	*	*	*	***	ns
Ag-I/L × Lend	ns	—	***	—	***	***	—	***	—
Ag-I/L × Lpart	*	—	***	—	ns	ns	—	***	—
Lend × Lpart	*	*	***	***	*	*	ns	***	ns
Ag-I/L × Lend × Lpart	*	—	***	—	***	***	—	***	—
hsd <sup>5</sup> value at 0.1%	0.29	0.31	4.5	2.6	11.4	7.2	9.8	6.7	

<sup>1</sup> Ag-I/L: First inflorescence that reached anthesis /young leaves or old leaves; the second inflorescence before anthesis/young leaves; Lend: Distal and Proximal end of leaves; Lpart: inner and outer part of leaf.

<sup>2</sup> Samples were not collected for analysis.

<sup>3</sup> Low<sup>10</sup> and high<sup>11</sup> based on the normal range of N (2.0–3.5%), B (30–100 ppm), Fe (50–300 ppm), and Mn (50–300 ppm).

<sup>4</sup>\*\*\*, \*, ns: significant at 0.1%, 5%, and non-significant, F-test.

<sup>5</sup> Honestly significant difference procedure.

symptoms that might be caused by Mn toxicity was not observed (Mastalerz, 1977).

The role of B in cell membranes in relation to boron phloem mobility and the boron-polysaccharide complex from cell walls is not well understood (Blevins and Lukaszewski, 1998; Dannel et al., 2002). Metabolic changes in cell walls or membranes of senescing leaves may further increase susceptibility to high levels of B. Boron is essential for cell wall structure (Brown et al., 2002). The level of B in most of tissues except in the outer part of the distal end of old leaves, when plants were fed with nutrient solution without B, was considered low based on the guidelines (Grace-Sierra Testing Lab, Allentown, PA). The level of B in these regions was also low even when plants were fed with fertilizer containing B (Roh et al., 2006). It is suggested that the lower limit of the normal range for B in *Curcuma* could be modified to about 10 ppm. This is significantly lower than the content in most greenhouse crops (minimum level of 15 ppm) except *Peperomia* or *Saintpaulia* (Hanan, 1998).

### Leaf-Margin Burn Symptom Expression in Relation to Boron (B)

High levels of B in the outer part of distal end of old leaves may not result from the translocation of B from the rhizomes via xylem translocation or passive absorption of B present in the medium, since the level of B in the whole rhizomes when harvested followed by nutrient solution lacking B was in the normal range for the medium, which may affect the passive diffusion dynamic (Brown et al., 2002). Therefore, it is not possible for B in the rhizomes and the growing medium to be absorbed, translocated, and deposited in the region with leaf-margin burn symptoms. Different levels of B present in various regions of leaves do not support the hypothesis that high levels of B are supplied from the rhizomes and growing medium. The B content increases from the lower to the upper part of the plant. The transpiration stream carries B to upper portions of the plant where it accumulates at the leaf margins and reaches high, toxic levels following evaporation of guttated water; this finally causes leaf-margin burn symptoms (Michael et al., 1969; Jones, 1970; Oertli, 1962). The low level of B (9 ppm) in the proximal end and edge of the old leaves, significantly lower than other parts, supports the concept that the source of B accumulated in the distal end and edge of the old leaves results from the translocation of B from other parts of the leaves.

Further studies using  $^{10}\text{B}$ -enriched boric acid are required to understand translocation of B (Shu et al, 1994). The difference in leaf-margin burn symptoms observed only in species with broad leaves could be related to the thickness of the cuticle layers, which must be examined. The difference in the amount of the droplets of guttation between species or cultivars with broad and narrow leaves should further be measured, and the content of B in droplets of guttate should be analyzed, if sufficient amount can be collected over the night, to

know whether the B level is actually higher than in the supplied water and in the tissue with and without showing leaf-margin burn symptoms.

## CONCLUSION

The source of B accumulated in distal end and outer part of the old leaves of 'CMU Pride' results from elevated B level following evaporation of guttated water droplets that could carry the dissolved B in the inner part of senescing leaves. Manganese may not be involved in leaf margin burn in *Curcuma* with broad leaves. The source of B accumulated in the outer region of the old leaves resulted from elevated B levels following evaporation of water droplets accumulated from guttation containing dissolved B in the inner part of leaves to the margin of senescing leaves. High levels of B in the edge of old leaves were likely not transferred from the rhizomes.

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